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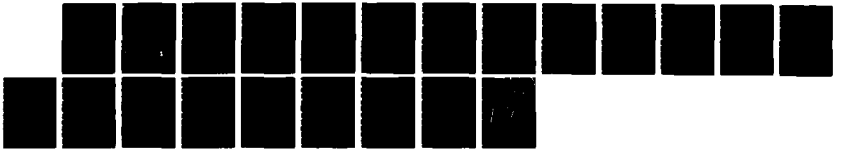

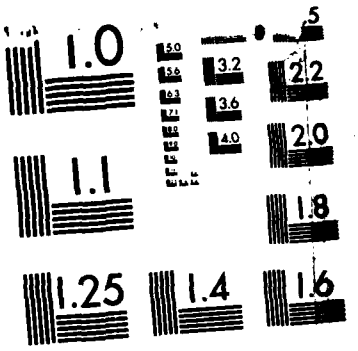

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FINAL TECHNICAL REPORT

to

OFFICE OF SCIENTIFIC RESEARCH

itle: The Metabolism of CIS - and Trans - Decalin in Fischer 344 Rats

rrincipal Investigator: M. Paul Serve', Professor of Chemistry

Contract Period: August 1, 1984 to July 31, 1985

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M. Paul Serve'
Professor
Department of Chemistry

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Male and female Fischer 344 rats were administered cis- and trans-decalin intragastrically. The male rats were more affected than the female rats as evidenced by reduced weight gain and appearances of hyalin droplet formation in the proximal tubules.
Urine studies in both male and female rats showed that cis-decalin was metabolized to cis, trans-1-decalol, cis, cis-1-decalol and cis, cis-2-decalol while trans-decalin was converted to trans, trans-1-decalol and trans, cis-2-decalol. Kidney extracts of male Fischer 344 showed the presence of 2-decalones.

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ABSTRACT

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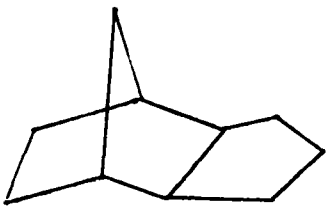
Male and female Fischer 344 rats were administered cis- and trans-decalin intragastrically. The male rats were more affected than the female rats as evidenced by reduced weight gain and appearances of hyalin droplet formation in the proximal tubules.

Urine studies in both male and female rats showed that cis-decalin was metabolized to cis, trans-1-decalol, cis, cis-1-decalol and cis, cis-2-decalol while trans-decalin was converted to trans, trans-1-decalol and trans, cis-2-decalol. Kidney extracts of male Fischer 344 rats showed the presence of 2-decalones.

Keywords: Decalin (decahydronaphthalene)

A study by Inman et al. showed that the Air Force Cruise Missile fuel JP-10, a C_{10} cyclic hydrocarbon with the chemical name exo-2,3,3a,4,5,6,7,7a-octahydro-4,7-methano-1H-indene (I), produced kidney lesions in Fischer 344 male rats. Using ^{14}C labeled JP-10, Inman found the hydrocarbon was distributed throughout the rat's body. A metabolic study of JP-10 showed the presence of the alcohol exo-5-hydroxy-exo-2,3,3a,4,5,6,7,7a-octahydro-4,7-methano-1H-indene (II) in the urine. Homogenization and extraction of the rat's kidney resulted in the isolation of the ketone, 5-keto-exo-2,3,3a, 4,5,6,7,7a-octahydro-4,7-methano-1H-indene (III). No ketone was found in the urine.



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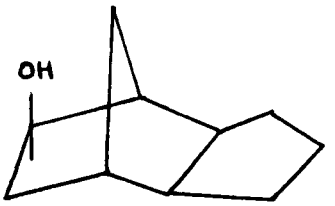
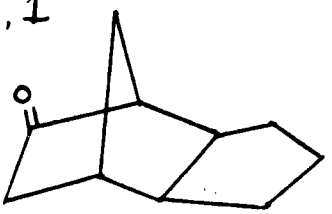


II

Based upon the work of Inman, it was hypothesized that the metabolites of high-boiling cyclic hydrocarbons may provide valuable information regarding the renal toxicity mechanism. It was proposed that the metabolism of other cyclic hydrocarbons be examined to see if a commonality of metabolism yielded similar toxic effects. The first chemical to be examined was decalin (IV).



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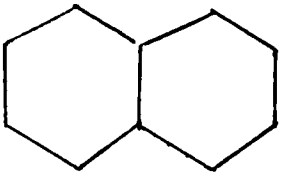


IV

Decalin (decahydronaphthalene) is an alicyclic hydrocarbon. Its principal use is as a solvent for oils, fats, and resins and as a turpentine substitute in oil paints. Decalin has also found use as a solvent and stabilizer in floor waxes and shoe creams where the mild turpentine odor can be easily hidden. Decalin is also a component of many fuel systems and was tested as a potential high energy density fuel for use in the submarine launched Cruise Missile system.

Decalin exists as two isomers (cis- and trans-) due to the stereochemical fusion of the two cyclohexane rings. The two isomers may be separated. Commercially purchased decalin consists of a cis/trans ratio of 54/46. The boiling point range of decalin is 188-195°C. The specific gravity is 0.885-0.890 and flash point is 57°C. The molecular weight of decalin is 138.

Previous studies with decalin have yielded the following information. Gage (2) found that exposure of 8 rats to 200 ppm decalin for 20 days on a 6 hour/day schedule yielded no toxic signs and grossly normal visceral organs at necropsy. Cardini (3) reported lung congestion, and kidney and liver damage in guinea pigs exposed to 319 ppm decalin for up to 23 days. Cardini also found that rabbits subjected to 319 ppm decalin died in 8-23 days, death being preceded by chronic convulsions. A human with prolonged exposure to decalin was found to have intense pruritus and vesicular eczema; the presence of albumin and leukocytes in urine also suggested kidney involvement. (3) In 1980, Gaworski et al. reported on the subchronic inhalation toxicity of decalin in

Beagle dogs, Fischer 344 rats, and C57B1/6 female mice. (4) Hematologic and clinical chemistry tests revealed no abnormalities attributable to decalin exposure in the dogs. Pathologic studies showed no gross or microscopic lesions in these dogs. The growth of male Fischer 344 rats was retarded by exposure to decalin vapors, but female Fischer 344 rats demonstrated little effect on weight gain. Lesions in rats were found only in males and were confined to the kidneys where 100% of the 5 ppm decalin exposure group and 96% of the 50 ppm decalin exposure group exhibited changes compatible with a toxic tubular nephrosis. The lesions, consisting of mild to moderate focal necrosis of proximal tubular epithelial cells with mild cystic tubular dilatation and intraluminal casts of granular, amorphous cellular debris at the corticomedullary junction, were dose related in severity. Female rats and control rats did not show these lesions. Mice presented lesions in the liver consisting mainly of fatty changes in the cytoplasm.

Recently, Phillips and Egan (5) have shown that exposure of male Fischer 344 rats to a paraffinic solvent containing hydrocarbons with a boiling point range of 156-176°C caused similar histologic kidney changes as those reported for decalin.

Investigations of the metabolism of decalin have been reported. Bernhard (6) reported that oral administration of mixed decalin isomers in dogs gave only an unidentified decahydronaphthol. Elliott et al. (7) found that cis-decalin given orally to rabbits was excreted as cis,cis-2-decalol (principal product) and cis, trans-2-decalol. Trans-decalin was excreted as trans,cis-2-decalol (principal product), trans, trans-2-decalol, and a trace of trans-1,2-diol. There is no report of a metabolic study of decalin in the rat.

EXPERIMENTAL:

The various decalols were prepared as follows. In those cases in which the decalol was prepared using a previously reported procedure, the procedure is referenced.

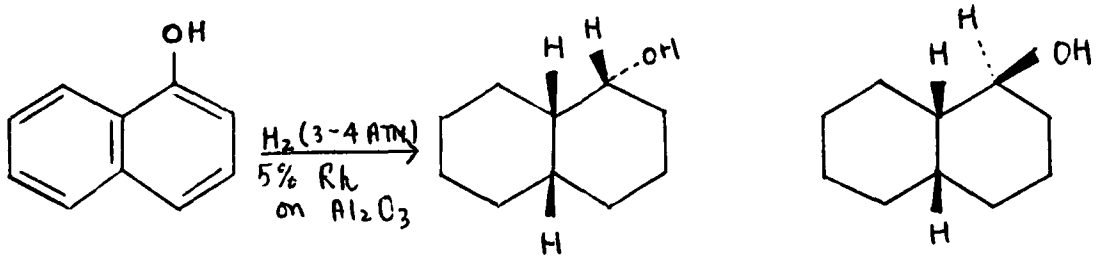
I. Preparation of Required Chemicals

CIS, CIS-1-DECALOL (IV) was prepared by the catalytic reduction of 1-naphthol (8). MP 92-3°C; lit MP 93°C.

CIS, TRANS-1-DECALOL (V) was isolated as a minor component of the catalytic reduction of 1-naphthol. Using thin-layer chromatography MP 64-5°C.

Anal. Calcd. for $C_{10}H_{18}O$: C, 77.87; H, 11.76

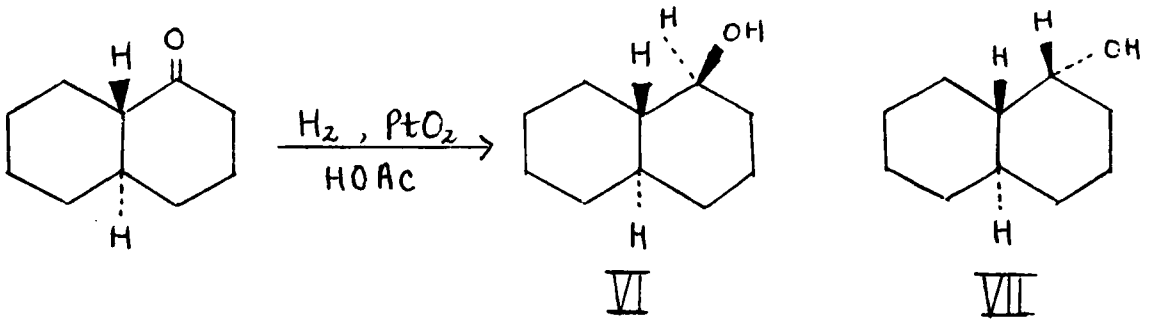
Found: C, 77.96; H, 11.64



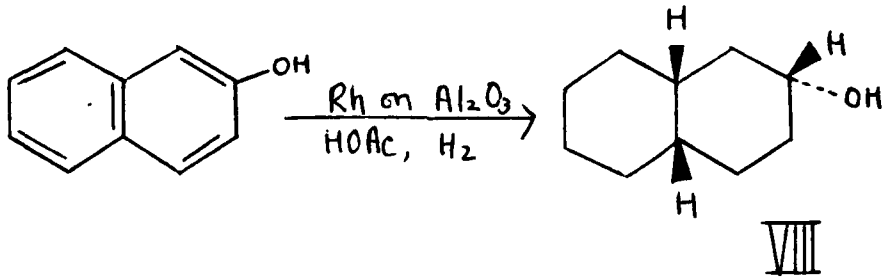
IV V

TRANS, TRANS-1-DECALOL (VI) was prepared by the catalytic reduction of trans-1-decalone using the procedure of Huckel. MP 58-9°C; lit MP 59-60°C. (9)

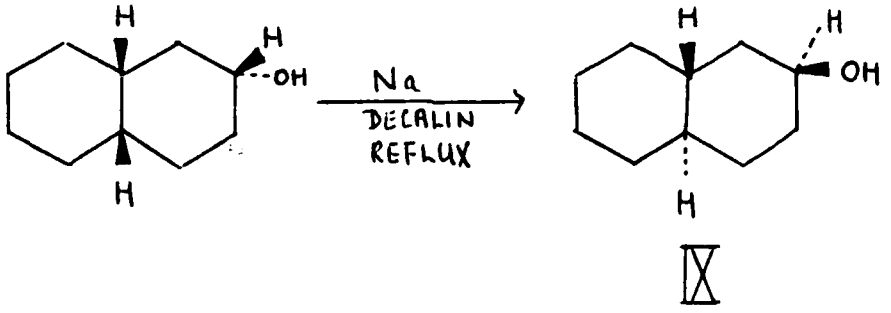
TRANS, CIS-1-DECALOL (VII) was isolated as a by-product from the reduction of cis-1-decalone using fractional crystallization. MP 48-9°; lit MP 49°C. (9)



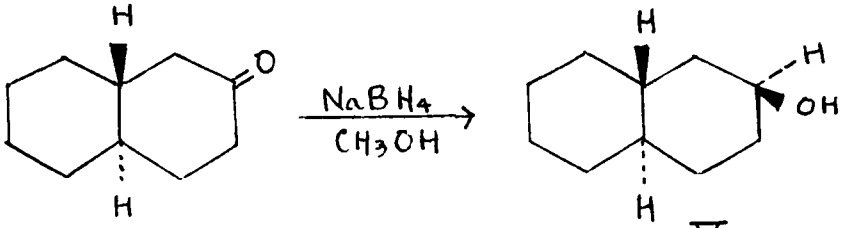
CIS, CIS-2-DECALOL (VIII) was prepared by the catalytic reduction of 2-naphthol using the literature procedure (10). MP 104-5°C, lit MP 105°C.



CIS, TRANS-2-DECALOL (IX) was obtained using the procedure of Rodig and Ellis (11). MP 18°C; lit MP 18°C.

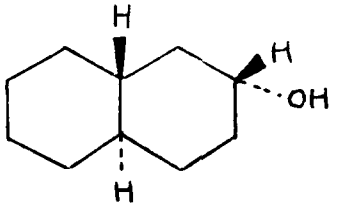


TRANS, TRANS-2-DECALOL (X) was prepared by the reaction of trans-2-decalone with sodium borohydride; MP 50-1°C; lit MP 50-1°C (12).



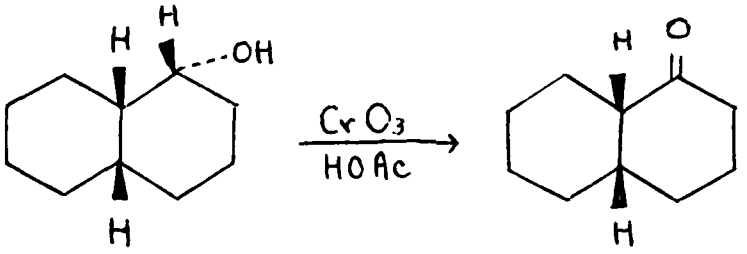
X

TRANS, CIS-2-DECALOL (XI) was purchased from International Flavors & Fragrances Inc., 521 West 57th Street, New York, NY, 10019; MP 73-4°C; lit MP 75°C (10).



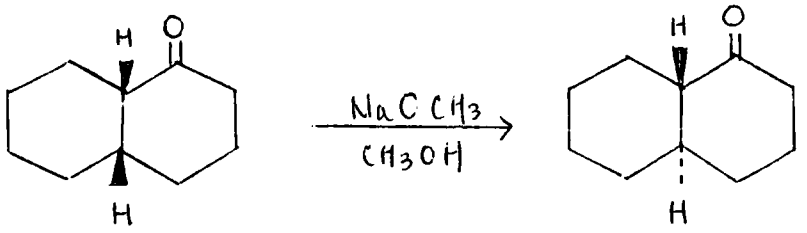
XI

CIS-1-DECALONE (XII) was prepared by the chromic acid oxidation of cis, cis-1-decalol; BP 127 (25mm); 2,4-dinitrophenylhydrazone MP 238°C; lit MP 238°C (10).



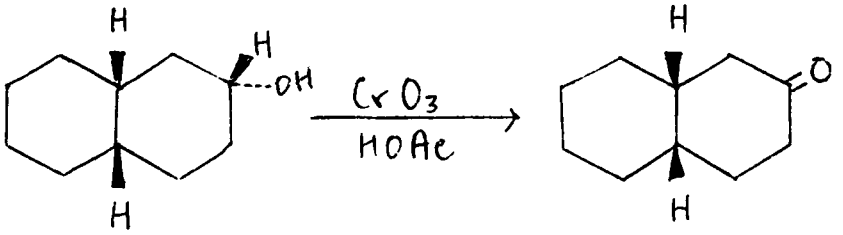
XII

TRANS-1-DECALONE (XIII) was formed by isomerizing cis-1-decalone with sodium methoxide; MP 30-2°C, lit. MP 32°C (10).



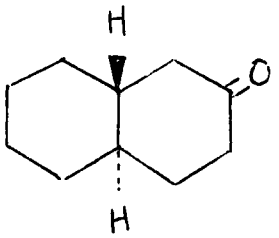
XIII

CIS-2-DECALONE (XIV) was prepared by the chromic acid oxidation of cis, cis-2-decalol; bp 115°C (20mm); 2,4-dinitrophenylhydrazone MP 154-55°C, lit MP 155°C (9).



XIV

TRANS-2-DECALONE (XV) was purchased from Aldrich Chemical Company, PO Box 2060, Milwaukee, Wisconsin, 53201.



XV

II. ANIMAL TESTS

Groups of male, female and control Fischer 344 rats with 7-9 rats/group were dosed intragastrically with 0.5 ml of cis-decalin, trans-decalin, or water (controls) on an every-other-day regimen for 14 days. The animals were placed in metabolism cages for two days after initial dosing so that urine samples could be collected.

During the 14-day dosing period, the animals were weighed daily. At the end of the dosing period, the animals were sacrificed and the livers and kidneys were removed for histopathologic studies. One kidney from each animal was saved for metabolite analysis, starting with homogenization of the kidney from each individual rat in saline solution. The kidney extraction samples (or urine samples) were then treated with the enzyme mixture beta-glucuronidase/aryl sulfatase (Calbiochem) at 37°C at a pH of 4.0 for 16 hours. Upon cooling to room temperature, the kidney extract or urine sample was then passed through a Clin-Elut column using methylene chloride as the elutent. The analysis scheme is outlined in figure 1. After evaporation, the samples were analyzed using a Hewlett-Packard 5880 gas chromatograph and a Hewlett-Packard 5985 gas chromatograph/mass spectrometer (GC and GC/MS conditions given in figure 2). The indentification of metabolites was accomoplished by matching GC retention times and MS fragmentation patterns of the metabolites with the GC retention times and MS fragmentation patterns of pure compounds previously prepared.

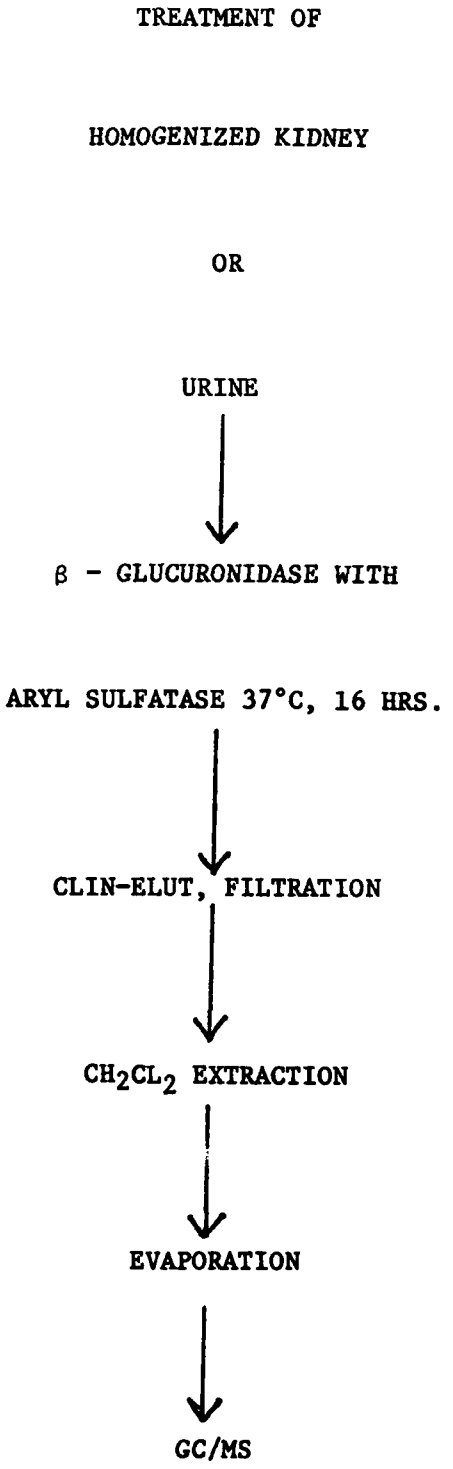


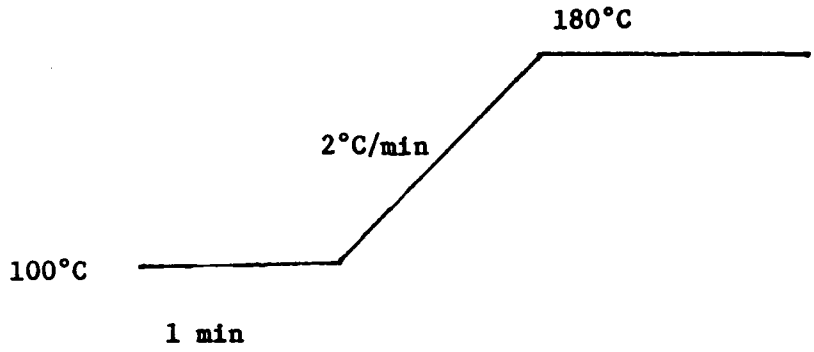
Figure 1. Analysis scheme for rat urine and rat kidney homogenate.

HP 5880 GC

CARBOWAX 20M CAPILLARY COLUMN 25 m x 0.2 mm ID

LINEAR VELOCITY 22 cm/sec at 100°C He

FID DETECTOR SPLIT RATIO 5:1 INJECTION PORT TEMPERATURE 200°C



HP 5985 GC/MS

5840 GC

3% SP-1000 4 ft x 2 mm ID GLASS COLUMN

FLOW RATE 28 ml/min He; INJECTION PORT TEMPERATURE 200°C

100/1/10/200/5

MS: QUADRUPOLE. EI MODE

ELECTRON ENERGY 70 ev

ION SOURCE TEMP 200°C

Figure 2. Gas Chromatography and Gas Chromatography/Mass Spectrometer Conditions for Analysis of Rat Urine and Kidney Homogenate.

RESULTS

A. Effect of Cis-Decalin and Trans-Decalin on the Weight of Fischer 344 Rats

The following tables list the average weights for rats dosed with either cis-decalin, trans-decalin or water (control) for the 14-day exposure period.

TABLE 1
Average Weight in Grams of Fischer 344 Male Rats Over The 14 Day Period. The Rats Were Dosed With 0.5 ml Cis-Decalin, Trans-Decalin or Water on Odd-Number Days.

MALE FISCHER 344 RATS

DAY	CIS-Decalin (7 Rats)	Trans-Decalin (6 Rats)	Water (7 Rats)
1	199	201	192
2	187	192	181
3	179	188	189
4	180	188	190
5	184	185	198
6	190	189	203
7	185	185	204
8	191	189	208
9	191	190	210
10	198	194	214
11	202	193	218
12	204	198	218
13	200	197	220
14	203	199	221

TABLE 2
Average Weight in Grams of Fischer 344 Female Rats Over The 14-Day Period. The Rats Were Dosed With 0.5 ml Cis-Decalin, Trans-Decalin or Water on Odd-Number Days.

DAY	CIS-Decalin (9 Rats)	Trans-Decalin (9 Rats)	Water (9 Rats)
1	148	152	147
2	142	147	151
3	146	151	150
4	145	152	152
5	151	156	155
6	150	154	156
7	155	158	158
8	158	158	160
9	154	161	160
10	154	157	160
11	155	159	160
12	154	160	162
13	158	161	162
14	160	162	163

Table 2 shows that the weight gain of female Fischer 344 rats was not affected relative to the control animals. During the initial dosing period, the female rats did suffer a weight loss which might have been due to eructation of the decalin which reduced appetite. During the second week of dosing, the effect of the decalin on weight gain seemed to be reduced.

Table 1 clearly demonstrates the deleterious effects that the decalins had upon weight gain of the male Fischer 344 rats relative to controls. During the first week of dosing, both decalins caused a significant weight loss, as in the case of the female rats. During the second week of dosing, the male rats adjusted to the decalins so that they regained the weight lost in week 1. However, unlike the exposed female rats which showed a definite weight gain, the male rats only returned to their starting weights. The male control rats showed an overall 16% weight gain. The male rats exposed to cis-decalin and trans-decalin showed a weight gain of 1% and 2.5%, respectively. The female control rats showed a 10.2% weight gain whereas those exposed to the cis-decalin and trans-decalin showed a 6.8% and 5.9% weight gain, respectively.

B. METABOLITES

The urine metabolites (with their relative abundance) isolated from the female and male Fischer 344 rats are listed in table 3.

TABLE 3.
Urine Metabolites Found in Female and Male Fischer 344 Rats
Treated With Cis- and Trans-Decalin.

DECALIN	METABOLITE	RELATIVE ABUNDANCE (GC Peak Areas)	
		Female Rat	Male Rat
CIS	Cis, trans-1-decalol	1.0	2.6
	Cis, cis-1-decalol		1.0
	Cis, cis-2-decalol	3.3	4.0
TRANS	Trans,trans-1-decalol		1.0
	Trans,cis-2-decalol	1.0	5.7

From Table 3 it can be seen that for both female and male Fischer 344 rats the principal metabolite of cis-decalin was cis, cis-2-decalol and for trans-decalin, the major metabolite was trans,cis-2-decalol. The difference in metabolism in the female and male rats is that treatment with cis-decalin yields cis,cis-1-decalol in the male which is not observed in the female. It was also noted that the metabolite cis, trans-1-decalol, although found in the urine of both male and female rats urine, was in larger quantities in the male (using the ratio of cis,trans-1-decalol/cis,cis-2-decalol). In the case of trans-decalin, the principal metabolic difference is the finding of the metabolite trans,trans-1-decalol in the male rat urine but not in the female rat urine. The analyses of the male Fischer 344 rat kidney extracts are listed in Table 4.

Table 4
Analysis of the Kidney Extracts from Male Fischer 344 Rats
Treated With Cis- and Trans-Decalin

DECALIN	NUMBER DOSED	METABOLITE	NUMBER CONTAINING NO KETONE
CIS	7	Cis-2-Decalone	0
TRANS	6	Trans-2-Decalone	1

The female Fischer 344 rats showed no decalin metabolites in their kidney extracts. Since ketones are normally formed from the corresponding alcohol, it was noteworthy that only the 2-decalones were found in extracts from male rat kidneys. The male rats had significant amounts of the 1-decalols in the urine but no 1-decalones were found in the kidney extracts.

A comparison of the metabolites of cis- and trans-decalin found in the rat with those found earlier in the rabbit (7) is interesting. The rabbit metabolizes the decalins only to the 2-decalols while male and female Fischer 344 rats yield both the 1- and 2- decalols. The finding of the cis- and trans- decalones in the male kidney extract appears to be unique. There was no report of the analysis of the rabbit kidney by Elliott.

Finally, analysis of both the male and female Fischer 344 rat urine without prior treatment with glucuronidase/sulfatase yielded no decalols. Thus, it appears that the decalols in the rat urine are excreted as glucuronic acid or sulfate conjugates.

C. HISTOPATHOLOGY

The 9 female Fischer 344 rats dosed with cis-decalin and sacrificed after 14 days had livers which were either normal or minimally affected (mild hepatocellular vacuolization in 6 rats). In addition, the kidneys of these treated female rats showed no recognizable lesions.

The 9 female Fischer 344 rats treated with trans-decalin and sacrificed after 14 days had livers which were either normal or had slight cytoplasmic vacuolization (4 rats). The kidneys of these trans-decalin treated female rats had either no recognizable lesions or minimal basement membrane proliferation (2 rats).

The 9 female Fischer 344 rats dosed with water had livers either normal or with minimal hepatocellular cytoplasmic vacuolization (4 rats) and the kidneys had no recognizable lesions.

All 7 male Fischer 344 rats dosed with cis-decalin had diffuse hyalin droplets ranging from mild to severe in the proximal convoluted tubules of the kidneys. Also found were multifocal casts of necrotic cells and debris near the corticomedullary junction.

Five of the 6 male Fischer 344 rats dosed with trans-decalin showed kidney damage with moderate to severe hyalin droplet accumulation in the proximal convoluted tubules and multifocal casts of necrotic cells and debris near the corticomedullary junction. The sixth rat had histologically normal kidneys.

The 7 male Fischer 344 rats treated with water showed minimal hyalin droplet formation in the proximal convoluted tubules of the kidneys.

The histopathologic data strongly indicate that both cis- and trans-decalin affect the male Fischer 344 rat more severely than the female rat. It is interesting to note that the 1 male rat showing no kidney damage with trans-decalin also did not have any trans-2-decalone in the kidney extract.

The finding of a ketone metabolite in the kidneys of male Fischer 344 rats treated with decalin as well as in the kidneys of rats treated with hydrocarbon JP-10 (I) implies that either the ketone is the cause of the kidney damage or that the ketone is a by-product of a biochemical reaction which arises as a result of the kidney damage.

The presence of the hyalin droplets in the proximal convoluted tubules indicates an inability to efficiently transport resorbed proteins from the glomerular filtrate to the capillary blood at the abluminal surface. Two pathogenic mechanisms, either alone or in combination, may be responsible for droplet formation. The first mechanism involves direct toxic injury to the tubular epithelial cells causing obstruction of protein transport and increased cytoplasmic accumulations. The other process results from glomerular disease in which excessive proteins leak into the glomerular filtrate and subsequently overwhelm the transport capacity of the tubular cells. Electron microscopic work indicates that the basement membrane, endothelial lining, and epithelial cell foot processes of glomeruli are normal.

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